Claims

1. A method for preparing an immunoglobulin binding protein array in plant cells, comprising the steps of:

(a) transforming a population of plant cells with a library of at least two different polynucleotides encoding different immunoglobulin binding protein (IgBP) polypeptides that:

(i) specifically bind to a ligand with a $K_D<10^{-6}$ moles/liter; or

(ii) form one or more disulfide bonds with one or more polypeptides in the transfected cell, to generate a binding protein that specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter;

wherein the IgBP polypeptides (i) comprise four framework regions alternating with three complementarity determining regions and (ii) comprise at least one peptide sequence having at least 75% sequence identity to a framework region of a native IgM, IgG, IgA, IgD, IgE, IgY, kappa or lambda immunoglobulin molecule; and wherein the IgBP polypeptides are not detectably expressed by the plant cells prior to transformation; and

(b) selecting transformed plant cells, and therefrom preparing an IgBP array in plant cells.

2. A method according to claim 1, wherein each IgBP polypeptide is a functional IgBP.

3. A method according to claim 1, wherein each IgBP polypeptide is an IgBP component that, upon disulfide linkage to one or more IgBP components encoded by other polynucleotides in the library, forms a functional IgBP.

4. A method according to claim 1, further comprising the step of:

(c) growing the transformed plant cells on a growth medium that supports replication of the plant cells, such that functional IgBPs are assembled by the plant cells.

5. A method according to claim 1, further comprising the steps of:

(c) growing the transformed plant cells on a growth medium to form plants; and

(d) sexually crossing the plants with themselves or other plants to generate progeny, such that the progeny comprise polynucleotides encoding IgBP components sufficient to form a functional IgBP.

6. A method according to claim 5, wherein the progeny are seeds.

7. A method according to claim 5, wherein the progeny are plants or plant cells that assemble functional IgBPs.

8. A method according to claim 1, wherein the library comprises at least 10 different polynucleotides.

9. A method according to claim 1, wherein the library comprises at least 100 different polynucleotides.

10. A method according to claim 1, wherein the library comprises at least 1000 different polynucleotides.

11. A method according to claim 1, wherein the library comprises at least 10,000 different polynucleotides.

12. A method according to claim 3, wherein the components comprise one or more portions of immunoglobulin molecules selected from the group

consisting of heavy chains and fragments thereof, light chains and fragments thereof, J chains and secretory components.

13. A method according to claim 1, wherein the polypeptides retain at least 95% amino acid identity to a framework region of a native IgM, IgG, IgA, IgD, IgE, IgY, kappa or lambda immunoglobulin molecule.

14. A method according to claim 1, wherein the framework regions are human.

15. A method according to claim 1, wherein the framework regions are murine.

16. A method according to claim 1, wherein the step of transforming is performed via *Agrobacterium*-mediated transformation, chemically-induced DNA uptake, electroporation, solid particle intrusion, biolistics, microinjection, macroinjection, lipofection or viral infection.

17. A method according to claim 1, wherein the IgBP polypeptides are secreted from the plant cells.

18. A method according to claim 1, wherein the plant cells are dicotyledonous plant cells.

19. A method according to claim 18, wherein the plant cells are tobacco or *Arabidopsis* plant cells.

20. A method according to claim 1, wherein the plant cells are monocotyledonous plant cells.

21. A method according to claim 20, wherein the plant cells are corn, Lemna or rice plant cells.

22. A method according to claim 1, wherein the plant cells are lower plant cells.

23. A method according to claim 22, wherein the plant cells are green algae cells.

24. A method according to claim 23, wherein the plant cells are *Chlamydomonas rheinhardtii*.

25. A method for preparing a heavy chain binding protein array in eukaryotic cells, comprising the steps of:

(a) transforming a population of eukaryotic cells with a library of at least two different polynucleotides, wherein each polynucleotide encodes a different heavy chain binding protein (C_HBP) polypeptide that:

(i) comprises an amino acid sequence that is at least 75% identical to a constant region tailpiece of a mu or alpha chain of a native immunoglobulin heavy chain;

(ii) comprises multiple combining sites, wherein all of the combining sites satisfy the same one of the following requirements:

(1) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin light chain variable region; or

(2) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin heavy chain variable region; and

(iii) either (1) specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter; or (2) forms one or more disulfide bonds with one or more polypeptides in the transfected cell, to generate a C_HBP that specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter; and

(b) growing the transformed cells on a medium that permits assembly of C_HBPs, wherein each C_HBP comprises at least four combining sites;

and therefrom preparing a C_HBP array in eukaryotic cells.

26. A method according to claim 25, wherein the polynucleotides encode immunoglobulin alpha or mu chains.

27. A method according to claim 25, wherein the cells are further transformed with one or more polynucleotides encoding polypeptides having sequences that are at least 75% identical to a sequence of an immunoglobulin J chain.

28. A method according to claim 25, wherein each C_HBP is assembled from four alpha chains and one J chain.

29. A method according to claim 25, wherein each C_HBP is assembled from twelve mu chains.

30. A method according to claim 25, wherein each C_HBP is assembled from ten mu chain and at least one J chain.

31. A method according to claim 25, wherein the C_HBPs or components thereof further comprise one or more portions of immunoglobulin molecules selected from the group consisting of J chains, secretory components and light chain constant regions.

32. A method according to claim 25, wherein the cells are plant cells.

33. A method according to claim 25, wherein the cells are insect cells.

34. A method according to claim 25, wherein the cells are mammalian cells.

35. A method for preparing a plant C_HBP array, comprising the steps of:

(a) transforming a population of plant cells with a library of at least two different polynucleotides, wherein each polynucleotide encodes a different C_HBP component that forms one or more disulfide bonds with one or more polypeptides in the transformed cell to generate a C_HBP that specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter, wherein each component:

(i) comprises an amino acid sequence that is at least 75% identical to a constant region tailpiece of a mu or alpha chain of a native immunoglobulin heavy chain; and

(ii) comprises multiple combining sites, wherein all of the combining sites satisfy the same one of the following requirements:

(1) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin light chain variable region; or

(2) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin heavy chain variable region;

(b) growing the transformed plant cells on a growth medium to form plants; and

(c) sexually crossing the plants to generate progeny, such that the progeny comprise polynucleotides encoding C_HBP components sufficient to form a functional C_HBP that comprises at least four combining sites;

and therefrom preparing a plant C_HBP array.

36. A method according to claim 35, wherein the progeny are seeds.

37. A method according to claim 35, wherein the progeny are plants or plant cells that assemble functional C_HBPs.

38. A method according to claim 25 or claim 35, wherein the library comprises at least 10 different polynucleotides.

39. A method according to claim 25 or claim 35, wherein the library comprises at least 100 different polynucleotides.

40. A method according to claim 25 or claim 35, wherein the library comprises at least 1000 different polynucleotides.

41. A method according to claim 25 or claim 35, wherein the library comprises at least 10,000 different polynucleotides.

42. A method according to claim 25 or claim 35, wherein the polynucleotides encode polypeptides that retain at least 95% amino acid identity to a contant region tailpiece of a mu or alpha chain of a native immunoglobulin heavy chain.

43. A method according to claim 25 or claim 35, wherein the step of transforming is performed via *Agrobacterium*-mediated transformation, chemically-induced DNA uptake, electroporation, solid particle intrusion, biolistics, microinjection, macroinjection, lipofection or viral infection.

44. A method according to claim 25 or claim 35, wherein the binding proteins accumulate in an intracellular compartment of the cells.

45. A method according to claim 25 or claim 35, wherein the binding proteins are secreted from the cells.

46. A method according to claim 32 or claim 35, wherein the plant cells are dicotyledonous plant cells.

47. A method according to claim 46, wherein the plant cells are tobacco or Arabidopsis plant cells.

48. A method according to claim 32 or claim 35, wherein the plant cells are monocotyledonous plant cells.

49. A method according to claim 48, wherein the plant cells are corn, Lemna or rice plant cells.

50. A method according to claim 32 or claim 35, wherein the plant cells are lower plant cells.

51. A method according to claim 50, wherein the plant cells are green algae cells.

52. A method according to claim 51, wherein the plant cells are *Chlamydomonas rheinhardtii*.

53. A C_HBP array in eukaryotic cells, comprising at least two eukaryotic cells that are each transformed with a different polynucleotide encoding at least one C_HBP polypeptide that:

(a) comprises an amino acid sequence that is at least 75% identical to a constant region tailpiece of a mu or alpha chain of a native immunoglobulin heavy chain;

(b) comprises multiple combining sites, wherein all of the combining sites satisfy the same one of the following requirements:

(i) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin light chain variable region; or

(ii) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin heavy chain variable region; and

(c) either (i) specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter; or (ii) forms one or more covalent bonds with one or more polypeptides in the transfected cell, to generate a C_HBP that specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter; and

(d) differs in amino acid sequence from other C_HBPs in the array;

wherein the cells assemble C_HBPs comprising at least four combining sites.

54. A binding protein array according to claim 53, wherein the polynucleotides encode polypeptide components of immunoglobulin molecules independently selected from the group consisting of heavy chains and fragments thereof, light chains and fragments thereof, J chains and secretory components.

55. A binding protein array according to claim 53, wherein the cells are plant cells.

56. A binding protein array according to claim 53, wherein the cells are insect cells.

57. A binding protein array according to claim 53, wherein the cells are mammalian cells.

58. A binding protein array according to claim 55, wherein the plant cells are selected from the group consisting of corn, rice, Lemna, tobacco and *Chlamydomonas*.

59. A binding protein array according to claim 53, wherein at least 10 different binding proteins are assembled by the cells in the array.

60. A binding protein array according to claim 53, wherein at least 100 different binding proteins are assembled by the cells in the array.

61. A binding protein array according to claim 53, wherein at least 100 different binding proteins are assembled by the cells in the array.

62. A binding protein array according to claim 53, wherein at least 10,000 different binding proteins are assembled by plant cells in the array.

63. A binding protein array according to claim 53, wherein each cell within the array is transfected with at least two different polynucleotides, each encoding a different C_HBP component, such that each cell assembles a functional C_HBP comprising the C_HBP components.

64. A composition comprising an array of encapsulated C_HBPs, wherein each C_HBP:

(a) comprises an amino acid sequence that is at least 75% identical to a constant region tailpiece of a mu or alpha chain of a native immunoglobulin heavy chain;

(b) comprises at least four combining sites, wherein all of the combining sites satisfy the same one of the following requirements:

(i) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin light chain variable region; or

(ii) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin heavy chain variable region; and

(c) either (i) specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter; or (ii) forms one or more covalent bonds with one or more polypeptides in a cell, to generate a C_HBP that specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter; and

(d) differs in amino acid sequence from other C_HBPs in the array.

65. A method for preparing a heavy chain binding protein array in eukaryotic cells, comprising the steps of:

(a) exposing multiple copies of a polynucleotide encoding a native heavy chain to a mutagen, such that random or site-directed mutagenesis of the polynucleotide occurs, resulting in a library of heavy chain variants;

(b) transforming a population of eukaryotic cells with the library of heavy chain variants; and

(c) growing the transformed cells on a medium that permits assembly of C_HBPs, wherein each C_HBP comprises at least four combining sites;

and therefrom preparing a C_HBP array in eukaryotic cells.

66. A C_HBP that:

(a) comprises an amino acid sequence that is at least 75% identical to a constant region tailpiece of a mu or alpha chain of a native immunoglobulin heavy chain;

(b) comprises at least four combining sites, wherein all of the combining sites satisfy the same one of the following requirements:

(i) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin light chain variable region; or

(ii) at least 75% identity to a 25 consecutive amino acid portion of an immunoglobulin heavy chain variable region; and

(c) either (i) specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter; or (ii) forms one or more covalent bonds with one or more polypeptides in a cell, to generate a C_HBP that specifically binds to a ligand with a $K_D<10^{-6}$ moles/liter.